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DNA Barcoding of Schistosome Cercariae Reveals a Novel Sub-Lineage within *Schistosoma rodhaini* From Ngamba Island Chimpanzee Sanctuary, Lake Victoria

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ABSTRACT: While *Schistosoma rodhaini* is typically considered a parasite of small mammals and is very scantly distributed in the Lake Victoria basin, it is known to hybridize with the more widespread *Schistosoma mansoni*, the causative agent of intestinal schistosomiasis. As part of broader parasitological and malacological surveys for *S. mansoni* across Lake Victoria, schistosome cercariae were harvested from a field-caught *Biomphalaria choanomphala* taken on Ngamba Island Chimpanzee Sanctuary, Uganda. Upon DNA barcoding, these cercariae were found to be a mixture of both *S. rodhaini* and *S. mansoni*, with further phylogenetic analysis revealing a hitherto unknown sub-lineage within *S. rodhaini*. Despite repeated sampling for eggs and miracidia from both chimpanzees and staff on Ngamba Island Sanctuary, detection of *S. rodhaini* within local definitive hosts awaits additional efforts, which should be mindful of a potential host role of spotted-necked otters.

While schistosomiasis is well recognized as a disease of major public health importance in sub-Saharan Africa (Stothard, Chitsulo et al., 2009), the significance of infections with other species of schistosomes, which more commonly infect animals, is often overlooked. While the latter schistosome species can be important in their own right, they have also recently begun to gather increased attention owing to reports of known or suspected hybridization with the human schistosomes, such as *Schistosoma mansoni* and *Schistosoma haematobium* (Morgan, DeJong, Lwambo et al., 2003; Huyse et al., 2009). Therefore, a better understanding of the epidemiology and evolutionary processes within animal schistosomes may be of both medical and veterinary significance.

With regard to *Schistosoma rodhaini*, this poorly known species is a sister taxon to *S. mansoni* and placed within the *S. mansoni* group (Morgan, DeJong, Kazibwe et al., 2003). *Schistosoma rodhaini* is commonly considered primarily an infection of rodents and insectivores (Hanelt et al., 2010). The species has a very patchy distribution in East Africa, where it co-occurs alongside the more widely spread *S. mansoni*; in areas of overlap, *S. mansoni* has been observed to be much more abundant, suggesting ecological dominance (Steinauer, Hanelt et al., 2008). This is perhaps due to the greater susceptibility of human hosts to *S. mansoni* compared with small mammals and *S. rodhaini*, which, for the latter, tend to have lower infection intensities (Steinauer, Mwangi et al., 2008; Hanelt et al., 2010). Intriguingly, at a single site in the Lake Victoria basin, genetic introgression between *S. mansoni* and *S. rodhaini* has been observed (Steinauer, Hanelt et al., 2008); several snails collected from this region near Kisumu in Kenya were co-infected with both species of schistosome (Steinauer, Mwangi et al., 2008). Elsewhere in Lake Victoria, a hybrid *S. mansoni* × *S. rodhaini* adult worm was recovered from a trapped rat on Ukerewe Island in Tanzania (Morgan, DeJong, Lwambo et al., 2003), which emphasizes the potential for interaction between these schistosomes given their close evolutionary relationship.

As part of a broader initiative to document the genetic diversity within *S. mansoni* in Lake Victoria, since 2008 we have surveyed for *Biomphalaria* spp. snails and schistosome cercariae from over 223 sites along the Lake Victoria shoreline in Uganda, Kenya, and Tanzania; schistosome-infected snails were generally rare (observed at only 9 sites), and, at each of these, cercariae were identified as *S. mansoni* using DNA barcoding (Standley et al., 2010; Standley, Vounatsou, Gosoniu, Jørgensen et al., 2012). Through the course of these expeditions, we additionally observed *S. rodhaini* at only 1 locality in Lake Victoria, specifically off Ngamba Island Chimpanzee Sanctuary in Uganda (Fig. 1). As had been observed at the sites in Kisumu, the *S. rodhaini* cercariae were isolated from a *Biomphalaria choanomphala* var. *choanomphala* snail (Standley, Wade, and Stothard, 2011) that was also co-infected with *S. mansoni*. Other parts of Ngamba Island were also surveyed, during 6 separate field expeditions throughout 2008–2011, and snails found to be infected with *S. mansoni* were observed at 3 other sites (Standley, Vounatsou, Gosoniu, McKeon et al., 2012); *S. rodhaini* cercariae were only observed once, at a single site (NG03), during a survey in June 2010. Ngamba Island is well known in this region for being a sanctuary for wild-born, re-homed chimpanzees (*Pan troglodytes*), which have recently been shown to be naturally infected with *S. mansoni* (Standley, Mugisha et al., 2011). Given the ecological uniqueness of the site, we used molecular methods to characterize the *S. rodhaini* collected from the island and compare it to other isolates of *S. rodhaini* as well as *S. mansoni*.

In the field, 29 individual cercariae harvested from the infected snail were preserved on a Whatman FTA® indicator card. DNA was extracted from individual spots using the method described by Gower et al. (2007). DNA barcoding was performed by sequence inspection of a partial segment of the cytochrome oxidase sub-unit 1 gene (COI) by PCR amplification with the primers ASMIT1 and ASMIT2, using standard thermal cycling conditions and cycle sequencing (Stothard and Rollinson, 1997; Stothard, Webster et al., 2009; Standley et al., 2010). The internal transcribed spacer region (ITS) of the nuclear genome was amplified from an additional 5 cercariae using ETTS1 and ETTS2 primers (Stothard et al., 1996) to cross check for putative hybrids. Amplified samples were purified using a QIAQuick PCR Purification Kit (QIAGEN Ltd., Crawley, U.K.). Sequencing reactions were performed on each purified PCR product using an Applied Biosystems Big DyeKit (version 1.1) (Carlsbad, California) and run on an Applied Biosystems 3730 DNA Analyzer.

Twenty-two cercariae were successfully sequenced for the COI ASMIT region and were visually aligned in MacClade 4 (Maddison and Maddison, 2005) and reduced to 5 unique *S. rodhaini* haplotypes (20 sequences) and 1 *S. mansoni* haplotype (2 sequences, which corresponded to an existing haplotype, with GenBank accession number AJ519524). The *S. rodhaini* sequences were submitted to GenBank and given the accession numbers JQ314100–JQ14104. The ITS sequences were identical and identified as *S. rodhaini* based on a BLAST search (www.ncbi.nlm.nih.gov/BLAST/), thus not suggesting the presence of hybrids. PAUP* 4.0 (Swofford, 2002) was used to create distance (neighbor-joining) and maximum parsimony trees using the 5 haplotypes plus *S. mansoni* COI sequences obtained from larval material from Ngamba Island and neighboring Kimi Island. Only 3 *S. rodhaini* sequences were available from GenBank that covered the ASMIT region of COI, and these were included in the phylogenetic analysis, along with 2 *S. haematobium*

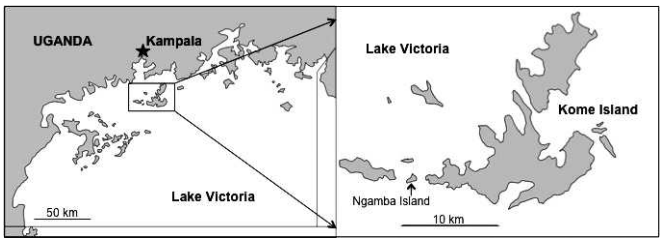

FIGURE 1. Map showing the location of Ngamba Island, where *Schistosoma rodhaini* cercariae were isolated from a *Biomphalaria choanomphala* var. *choanomphala* snail. The geographical positioning system coordinates for the site are 0.10095°S, 32.65538°E.

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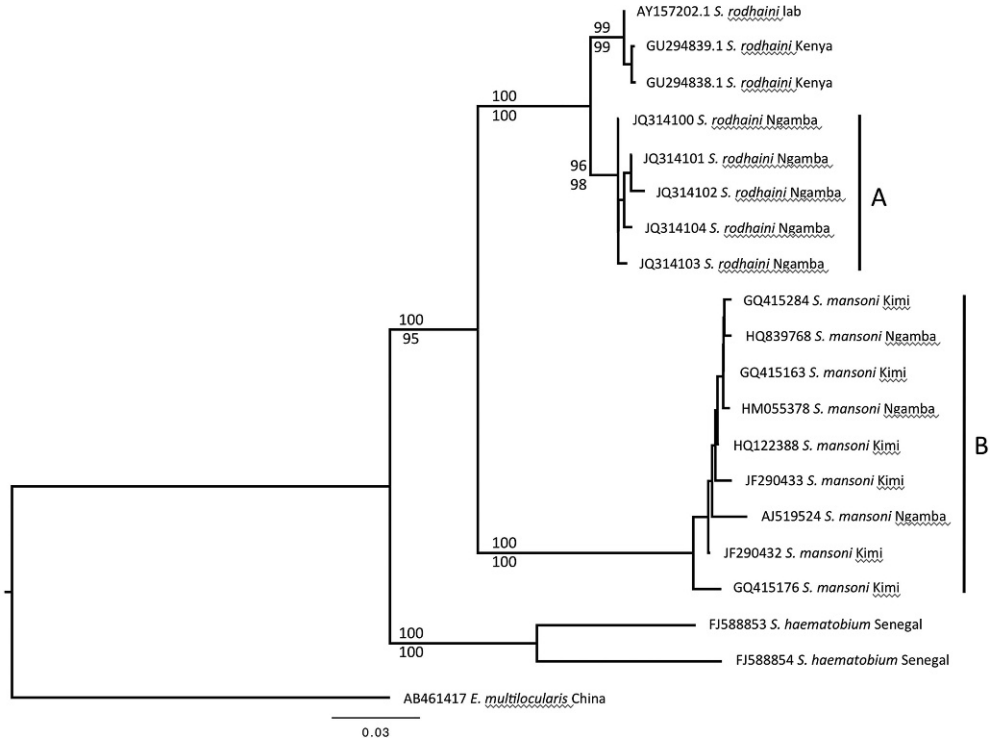

FIGURE 2. Phylogenetic tree showing position of Ngamba Island *Schistosoma rodhaini* samples (clade "A") relative to sympatric haplotypes of *Schistosoma mansoni* (clade "B") and GenBank deposited sequences of *S. rodhaini*, *Schistosoma haematobium,* and *Echinococcus multilocularis*. Tree topology is based on distance (neighbor-joining) method; the numbers above and below the node refer to bootstrap values from maximum parsimony and neighbor-joining, respectively (1,000 replicates each). Labels show GenBank accession number, the species name, and the collection location.

sequences from GenBank and a sequence of *Echinococcus multilocularis* as an outgroup. Node support was estimated using bootstrapping (1,000 replicates for both methods).

The resultant tree showed strong support for *S. mansoni* as a sister group to the *S. rodhaini* clade, as expected. In Figure 2, "A" designates the *S. rodhaini* sequences from Ngamba Island, whereas "B" shows sympatric *S. mansoni* sequences. Within the *S. rodhaini* clade, the sequences from Ngamba Island formed a distinct, well-supported sub-clade to the existing laboratory and field-collected isolates of *S. rodhaini*. However, branch lengths were not long, suggesting low levels of differentiation and a recent split between the groups. Uncorrected *p*-distance estimates within the 2 *S. rodhaini* clades were 0.002 for the GenBank samples and 0.007 for the Ngamba sequences; the distance between the clades was 0.022, which was greater than the distance of the *S. mansoni* samples from either lineage included in Figure 2, and supported the conclusion that the Ngamba samples constitute a sub-lineage of significant differentiation from other *S. rodhaini* haplotypes. The presence of a novel sub-lineage of *S. rodhaini* could be accounted for by the relative isolation of Ngamba Island, both geographically and ecologically, given its status as a protected sanctuary among fishing communities with a high human population density.

From an alternative perspective, it could also be suggestive of novel epidemiological opportunity and putative changes to the transmission of *S. rodhaini* occurring on Ngamba. For example, there are suspected populations of introduced, as well as native, rodents on Ngamba Island; previous trapping efforts of rodents have not been successful, so it is unknown which species may be acting as terminal host in this environment. Despite repeated sampling over several hundred miracidia from chimpanzees and local staff members resident on the island, the detection of *S. rodhaini* infections awaits additional efforts, with only *S. mansoni* found so far. However, it is worth noting that baboons already infected with *S. mansoni* are at greater risk of *S. rodhaini* infection. Thus, continued efforts should be made to monitor the possibility of co-infection in resident primates on Ngamba (Nelson and Teesdale, 1965). Other mammals could be responsible for the introduction and local maintenance of transmission, such as the resident population of spotted-necked otters (*Hydrictic maculicollis*); a haul-out site, with fresh scat, was observed close to where the snail infected with *S. rodhaini* was collected. Another factor of interest is that spotted-necked otters are becoming ever more threatened by habitat destruction elsewhere in Lake Victoria, and so the exposure to novel pathogens such as *S. rodhaini* may have implications for their local conservation.

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